## PSYCHE.

## SOME EXPERIMENTS ON FLACHERIE IN THE GYPSY MOTH.1

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In Volume XXVI, Nos. 13, 14, 15, and 16 of the "Biologisches Centralblatt" (Leipzig 1906) Dr. E. Fischer of Zürich has published some important studies on the susceptibility of caterpillars to diseases. In his investigations he has taken extraordinary care in establishing the primary causes leading to the disease variously known as "flacherie," "flaccidenza" and "caterpillar-cholera," a disease which deserves special attention on account of its extreme infectiousness. He has pointed out that the first tendency toward the disease lies in a decrease in the nutritive value of the food of the caterpillars, which suddenly induces a disturbance in metabolism. As a result the organisms responsible for the disease immediately find conditions suitable for their growth. One of the main causes of the disease is therefore to be sought in the susceptibility of the caterpillars, while the specific infection of flacherie is to be regarded as coming more or less secondarily; in other words, without susceptibility infection cannot take place, and this susceptibility can be brought about by insufficient nourishment. In his experiments Fischer produced the proper susceptibility by giving the caterpillars food which he placed in water and renewed only every three or four days. This treatment causes an injury to the leaf protoplasm resulting from the presence of too much water. Before the visible outbreak of the. disease he could recognize as an early symptom, a characteristic sweet odor in the breeding cages, which could be compared to that of withered lilac blossoms. Whenever this odor was noticeable flacherie soon made its appearance and as it progressed the odor increased proportionately. It is a well known fact that in the occurrence of Psilura monacha, the Nun, ("die Nonne" of the Germans) in unusual numbers in Germany the only relief has come through the outbreaks of flacherie. Fischer therefore recommended, after the completion of his experiments, the artificial production of flacherie by intentionally giving the larvæ poor food as soon as the abundance of Psilura monacha was noted in any particular locality.

<sup>&</sup>lt;sup>1</sup> Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University, No. 7.

Bearing in mind the close relationship existing between this pest and the Gypsy Moth, and realizing that, in the entire animal world, one of the greatest checks to overproduction is the appearance of epidemic diseases, I attempted to put Fischer's conclusions regarding the artificial production of flacherie, to a practical test. This was done by the following series of experiments on the Gypsy Moth. Several thousand caterpillars from normal overwintered eggs were reared, from the time of hatching, under the best possible conditions, each egg-mass being kept separate. Immediately after the second molt, the larvæ were removed, with the exception of small control series, and those from each egg-mass divided among isolated trees, in such a manner that each tree received caterpillars from only a single egg-mass. The isolation of the individual trees was accomplished by means of a board ring about ten centimeters high, smeared with tanglefoot to prevent the caterpillars from crawling over, and large enough to include all perpendicular lines dropped from the tips of the outermost branches. Previously, of course, each tree had been carefully cleared of any foreign caterpillars, and care was taken to utilize only such trees as had been only slightly or not at all damaged by caterpillars the previous year. Oaks, birches and apple trees were used. About four days after the second molt fifty caterpillars altogether were removed from these various trees and placed in a small breeding cage. There they were fed on their normal food plant, oak, but upon leaves which had been previously soaked with the attached small twigs in water for four days. After six days of such feeding, I recognized the sweet odor which is the early symptom of flacherie, and two days later the first dead caterpillars were to be seen. Two days later still, I counted twenty dead specimens, which I distributed, together with those still living, upon three isolated trees (oak, apple and birch). On the next day the first death on these trees occurred, and in this experiment by the time of pupation, the mortality had reached fifty-five to sixty percent.

In a similar way a second experiment was made, but with caterpillars which had not yet passed the fourth molt. Among these, the early symptoms of the disease were noticeable as early as the second day, and the first dead specimens were to be found two days later. With the dead and diseased larvæ of this series, the caterpillars of the same age on three trees (oak, apple and birch) were infected. In this case the disease made more rapid headway, but the proportion of dead specimens at the time of pupation exceeded that of the first experiment by only five percent, that is to say, it amounted to sixty-five percent.

A third experiment was then undertaken in the following way: to twenty-five caterpillars, taken just after death at which time they turn into a thin semi-liquid or jelly-like mass, two liters of water containing a small quantity of glue were added.

With this mixture the trunks of three trees (oak, birch and apple) were painted with a ring about ten em. in width, placed right under the lowest larger branches. The caterpillars on these trees had just passed the third molt. At the end of three days the first dead specimens were found, after which the disease spread in the same manner as in the second experiment. At the time of pupation about sixty-three percent of this series of larvæ had fallen a prey to the flacherie.

As a fourth experiment, a mixture similar to the one just described was used and sprayed on the foliage of three more trees including oak, birch and apple, by means of a small "Spramotor" sprayer. To make the spray adhere better to the leaves a small quantity of glue was added. In this case also the larvæ had recently undergone the third molt. At the end of two days the first dead specimens were observed and their number very rapidly increased from day to day, until at the time of pupation about seventy percent had succumbed to the disease.

In a fifth experiment twenty dead larve were carefully dried, powdered in a mortar and then stirred into three liters of water. With this decoction three isolated trees were sprayed in the same manner as before, but this time the results were not very satisfactory, as before pupation only about forty percent of the larve died. In this case the experiment also was begun after the caterpillars had already passed the third molt. It is possible that the lower percentage of deaths may have been due to the addition of too much water to the dried material.

In all these experiments the still remaining pupe were later looked for and it was found that an average of from ten to fifteen percent had died from flacherie. It must be expressly stated that the trees used in the experiment were regularly watered and their leaves sprinkled with ordinary tap water twice a week to counteract the effects of the unusually dry summer of 1909, and to keep them as nearly as possible in a normal condition. It is also to be noted that the possibility of transfer of the flacheric from one tree to another through the agency of wind was well-nigh precluded as only very slight winds occurred here, and besides, the trees treated were far removed from one another. Neither were any of the trees supplied with an unusually large number of larvæ in order that the possibility of contact between them might not be made abnormally probable.

All caterpillars which were kept apart for control remained healthy. The control was managed in the following way. Ten eaterpillars coming from each separate egg mass used in the experiments were separated after the second molt and each lot kept apart with great care. These were reared out of doors in special breeding eages which altered as little as possible the external conditions. They were fed on leaves from a small oak tree, especially selected for the purpose, which had been nearly free

from caterpillars the preceding year. In addition, the tree was very carefully protected from any foreign caterpillars or insects, and was carefully watered like the other trees used in the experiments. The food was regularly renewed each morning and evening, and the caterpillars themselves were sprayed one morning in each week with ordinary tap water just before they were given fresh food.

From the foregoing results I have therefore been led to believe that the artificially produced flacherie can be utilized as a valuable aid in the destruction of Gypsy Moth caterpillars. As is well known, the disease commonly appears in nature only after the caterpillars are full grown, and even then only during unusually dry or very damp seasons. The fact that I have now succeeded in rendering the caterpillars susceptible to flacherie before the third molt (Experiment 1.) may be of importance for the practical use of the disease, since by artificially inducing flacherie, relief might be had weeks sooner than happens in nature. In addition to this my experiments admit of the conclusion reached by Suzuki who experimented on mulberry trees in Japan. (Cf. "Zeitschrift f. Pflanzenkrankheiten," XII. Band, Jahrg. 1902, 4. Heft p. 203-226, 5 Heft p. 258-278.) Suzuki found that in the case of insufficient nourishment there was also a concomitant increase in the acidity of the leaves (p. 272). If this be the case, it would seem that the alkaline reaction of the digestive juices must be neutralized in order to bring on the first susceptibility of the disease. Full grown caterpillars are most readily affected because at each stage of their growth the alkalinity of their digestive fluid decreases. Normal young caterpillars have a strongly alkaline fluid, which, according to the researches of Verson and Bolle, has the power to destroy the "polyhedric corpuscles" which are very resistant to disinfectants (Fischer, p. 542). That the alkalinity of the digestive juices of the young caterpillars actually suffers a decrease through insufficient nourishment is indicated by my experiments, since in these cases the young caterpillars succumbed to the disease. Had the alkaline content of the digestive juices not decreased, the pathogenic organisms should not have shown increased virulence.

In addition to the five experiments previously described, a sixth one was undertaken in an open field containing a group of oak and another of willow trees each of which was infested with about five thousand caterpillars. Upon each of these two groups of trees one hundred sick caterpillars and fifty dead ones were distributed. The larvæ were ready for the fourth molt at the beginning of the experiment, and even on the next day the count of dead specimens could be begun. The disease spread with amazing rapidity till by the time of pupation about four thousand caterpillars on each group of trees had succumbed. Two conditions, which did not enter into my previous experiments, united to cause this unusual result. In the first place,

the two groups of trees had been badly infested and injured by Gypsy Moth caterpillars the previous season, as was evidence by the unhealthiness of the leaves during the summer of 1909, and the caterpillars had become predisposed to the disease on account of the resulting decrease in the value of their food. As a second very important factor may be mentioned the extremely dry weather, which by its desiccating effect on the leaves served to render the food for the caterpillars still worse. On this account, from the beginning there was a decreased vitality in the tissues and digestion was disturbed. In short, the caterpillars were already very susceptible to the disease at the time I introduced it among them. Conditions were therefore most suitable for the spread of flacherie. Apparently in the places that have been injured by Gypsy Moth caterpillars in previous years, there is also a predisposition on the part of caterpillars of the following year toward flacherie, and the organisms of the disease from the introduced dead and sick caterpillars will act readily upon the more healthy ones. Infection will take place even in cases where a locality is first badly infested by the caterpillars, because one can always find a large number of weakly specimens which lend themselves more readily to treatment. Then, as the disease progresses, it acquires such virulence that the previously healthy specimens become susceptible. If, however, unusual climatic conditions prevail the disease will find the environment already suitable for its dissemination. This ought to be particularly true, if the organisms causing the disease should be fungi. In the production of a purely bacterial disease the climatic conditions would hardly play such a highly important rôle.

There are some other peculiar habits of the larvæ which may be important factors in the spread of the disease under conditions like those of the third experiment. Since the caterpillar is very active just before beginning to feed, and since it likes, when half or full-grown, to crawl up and down the tree trunk, it is forced to pass over the ring surrounding the trunk and infection results. Serving also to hasten their destruction is their peculiar desire to suck the juices of other caterpillars which have died of flacherie. They are also very fond of sucking the ring placed on the tree and so imbibe directly the diseased material. In cases of extraordinarily dry weather one may renew the ring after about two days. The probability of infection between caterpillars themselves is also always very great. The disease is transmitted particularly during resting periods, for it very commonly happens that the caterpillars become gregarious at such times. Then the disease continues to spread when a healthy caterpillar feeds on that part of a leaf which has been previously tasted by a diseased one. The excrement of the diseased caterpillars is very moist on account of their digestive disturbances, and this infected matter commonly adheres to the leaves and twigs, where it readily comes in contact with healthy caterpillars, not to mention the fact that it is seized upon with avidity by the caterpillars as long as it remains moist. Sick caterpillars also exude from the mouth liquids which may infect healthy specimens through the habit just mentioned.

In this connection certain other investigations made by Fischer also deserve notice. He found that caterpillars when sick with flacherie, if not too far gone, and if they are still able to feed, can be cured if they be separated from those already dead and given the very best of care, with extremely fresh food renewed possibly two or three times a day. During convalescence the peculiar sweet odor noticeable as an early symptom of flacherie disappears. I repeated Fischer's experiments with Gypsy Moth caterpillars after the third and fourth molts with the same successful result. This is of especial value to caterpillar breeders, for it often happens that whole series of larvæ fall a prey to flacherie, without their knowing of any way to stop its progress. Further there is the erroneous opinion that in large breeding series the disease is very apt to break out spontaneously, but this, however, is not at all likely if fresh, sound food is given at least twice a day and the natural life conditions of the caterpillars are reproduced.

I must not forget to call especial attention to the fact that all the experiments here briefly described have required a great deal of time and attention. One must carefully look after every detail of the experiments daily from the time of the hatching of the Gypsy Moth eggs until pupation (from the end of May till the end of July) in order to avoid even slight mistakes or errors of apparently minor importance which might lessen the value of the work. One must also consider the various possibilities which count for or against the practical use of artificially produced Flacherie.

Although my experiments suggest the great probability of an economic value in this disease for destroying the Gypsy Moth, I must make it plain that the experiments of only a single year had best not be taken as a comprehensive method for the practical use of Flacherie, but that further experiments should be undertaken on a larger scale in the near future to substantiate the results I have obtained.

It goes also without saying that such work should be done only with great care to secure correct results, for it does not depend on the *performance* of experiments, but above all on *how* they are performed.

I may also mention that I performed experiments with the brown-tail moth (Euproctis chrysorrhaa), similar to those performed with the gypsy moth, but in this case successful results were not obtained, as only two percent of the caterpillars succumbed to the disease. I have, moreover, observed a similar percentage of dead chrysorrhaa caterpillars in nature near Raymond, N. H. There I found a wooded area where flacherie had broken out especially among the American tent caterpillars

(Malacosoma americanum Fabr.) and those of various Noctuidae, but in spite of the presence of brown-tail caterpillars in large numbers a mortality of only two percent by flaeherie appeared among them. It is possible that the caterpillars of this species on account of the larger amount of tannin which they contain, may be almost immune against the organisms of flacherie and it may have been only the weakest individuals which fell a prey to the disease.

Whether flacherie is hereditary or not has not yet been positively established. Standfuss (Handbuch der palæarktischen Gross-Schmetterlinge, 2. Auflage, Jena, 1896) leans toward the latter conclusion, assuming that infected caterpillars never survive until the adult stage (p. 160). Experiments would, however, be necessary to prove this hypothesis, for as already mentioned, it has been shown that caterpillars affected with flacherie can pupate, later dying in that stage. And further, in my experiments on *Junonia cania* (Journ. Exper. Zool. Vol. VI, No. 4, June 1909, pp. 13) this was shown where (p. 555) I referred to a pupa in which the body was decomposed by flacherie. It had died about six hours before the emergence of the butterfly, since all the parts of the specimen were entirely developed. The possibility of the inheritance of the disease cannot be cast aside therefore without further data.

As to the actual primary cause of flacherie but very little can as yet be stated with certainty. Fischer (l. c.) thought that several species of micro-organisms (bacilli) are concerned in the disease, while Dr. Hofmann (Insektentötende Pilze, Frankfurt, a. M. Peter Weber Verlagshandlung) as early as 1891 found present in the excrement and body cavities of caterpillars affected with the disease, extremely small bacilli, innumerable schizomycetes, and more particularly small strings of micrococci. The relationship of these micro-organisms to flacherie are still to be elucidated by the investigations of bacteriologists and pathologists.

I am especially indebted to Prof. W. M. Wheeler for many suggestions given me in the preparation of the foregoing account and for the encouragement he has given me in the work.

Bussey Institution, October 10th, 1909.